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Mutation rates, spectra, and genome-wide distribution of spontaneous mutations in mismatch repair deficient yeast

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Sequencing data are available through NCBI (SRA Study Accession Number SRP026313).

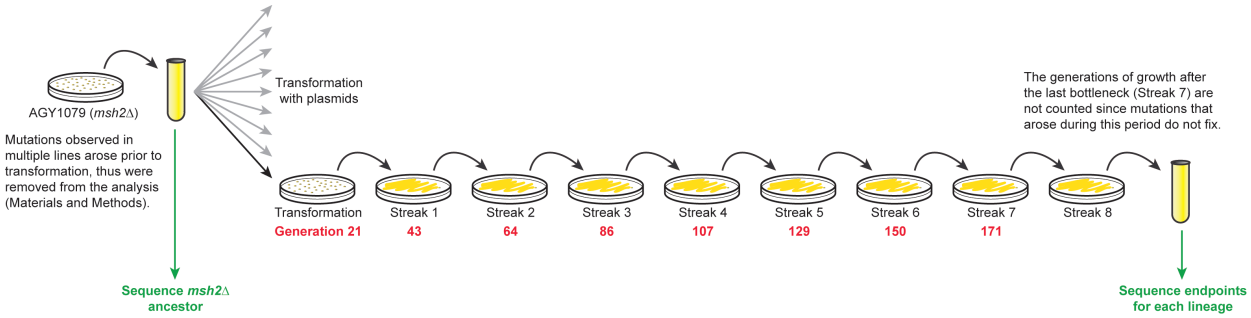
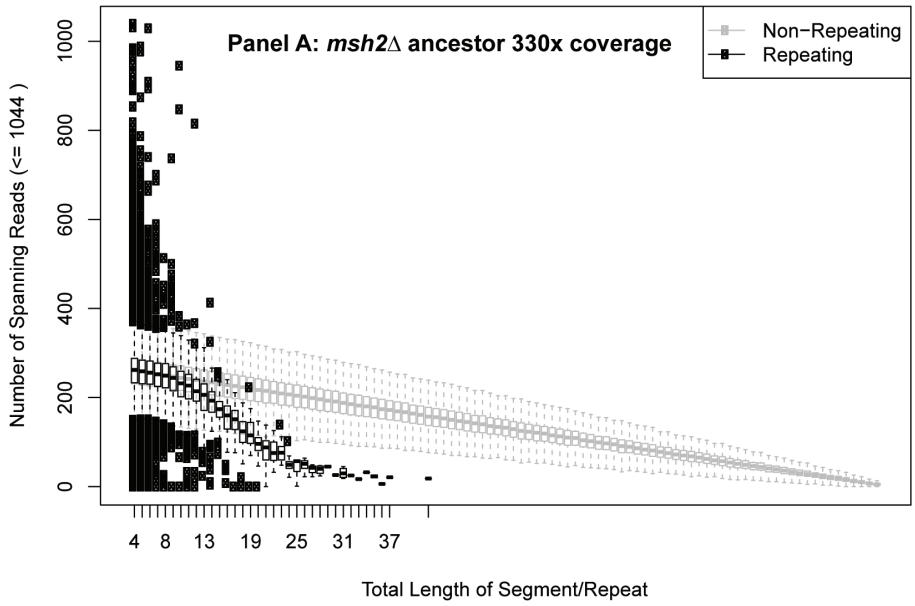


Figure S1: Schematic of Experimental Design. A single colony of AGY1079 was picked and transformed with *msh2*-containing plasmids. A single colony from each transformation was selected to begin the mutation accumulation experiment. Strains were passaged by streaking for single colonies every two days. We estimate ~21 generations of growth between bottlenecks.



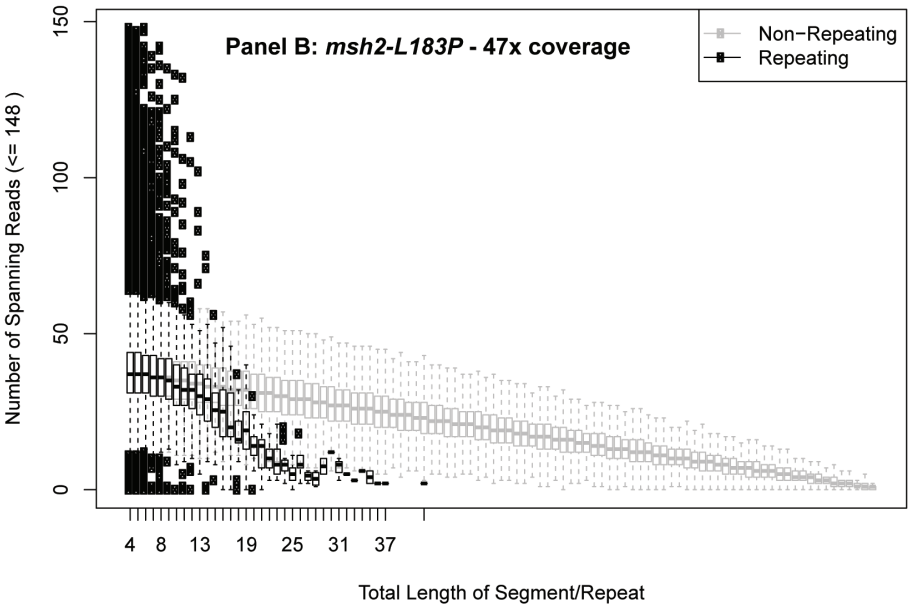


Figure S2 Spanning Read Counts for Repeats

Bedtools intersectBed (see Materials and Methods) was used to find the number of reads that overlap a microsatellite (repeating) region as well as non-repeating regions of varying length. Regions from chromosome XII (rDNA repeats) as well as regions with a read count >= 4x median were removed before plotting.

Table S1 Plasmids used in the study

Strain*	Plasmid Name	Relevant Genotype	Published Source
	pRS413	*HIS3 CEN/ARS*	(Sikorski and Hieter 1989)
AG17	pMSH2	*MSH2 HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG35	pMSH2-G688D	*msh2-G688D HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG86	pMSH2-D524Y	*msh2-D524Y HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG403	pMSH2-G770R	*msh2-G770R HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG421	pMSH2-A618V	*msh2-A618V HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG424	pMSH2-S742F	*msh2-S742F HIS3 CEN/ARS*	(Gammie *et al.* 2007)
AG486	pMSH2-L183P	*msh2-L183P HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG487	pMSH2-P640T	*msh2-P640T HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG488	pMSH2-P689L	*msh2-P689L HIS3 CEN/ARS*	This study
AG495	pMSH2-C195Y	*msh2-C195Y HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG496	pMSH2-R542L	*msh2-R542L HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG497	pMSH2-D621G	*msh2-D621G HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG499	pMSH2-S695P	*msh2-S695P HIS3 CEN/ARS*	This study
AG507	pMSH2-C345F	*msh2-C345F HIS3 CEN/ARS*	(Arlow *et al.* 2013)
AG545	pMSH2-R657G	*msh2-R657G HIS3 CEN/ARS*	This study
AG546	pMSH2-G693R	*msh2-G693R HIS3 CEN/ARS*	This study
AG547	pMSH2-T743K	*msh2-T743K HIS3 CEN/ARS*	This study

*****All plasmids from the Gammie laboratory (Princeton University) except for pRS413

Table S2 Sequencing Coverage

Relevant Genotype	Description	Generation	Coverage
MSH2	genomic WT ancestor	0	299 x
MSH2	genomic WT passaged	~170	194 x
*msh2*Δ	Null ancestor	0	330 x
*msh2*Δ + pMSH2	CEN WT passaged	~170	165 x
*msh2*Δ + pRS413	Null passaged	~170	76 x
*msh2*Δ + pMSH2-L183P	Allele passaged	~170	47 x
*msh2*Δ + pMSH2-C195Y	Allele passaged	~170	147 x
*msh2*Δ + pMSH2-C345F	Allele passaged	~170	133 x
*msh2*Δ + pMSH2-D524Y	Allele passaged	~170	220 x
*msh2*Δ + pMSH2-R542L	Allele passaged	~170	145 x
*msh2*Δ + pMSH2-A618V	Allele passaged	~170	94 x
*msh2*Δ + pMSH2-D621G	Allele passaged	~170	255 x
*msh2*Δ + pMSH2-P640T	Allele passaged	~170	103 x
*msh2*Δ + pMSH2-R657G	Allele passaged	~170	118 x
*msh2*Δ + pMSH2-G688D	Allele passaged	~170	166 x
*msh2*Δ + pMSH2-P689L	Allele passaged	~170	188 x
*msh2*Δ + pMSH2-G693R	Allele passaged	~170	60 x
*msh2*Δ + pMSH2-S742F	Allele passaged	~170	153 x
*msh2*Δ + pMSH2-T743K	Allele passaged	~170	192 x
*msh2*Δ + pMSH2-S695P	Allele passaged	~170	99 x
*msh2*Δ + pMSH2-G770R	Allele passaged	~170	175 x

Table S3 Parameters for Mapping with BWA for Illumina

Input Parameter	Value
Conditional (genomeSource)	0
Select a reference genome	w303_draft
Conditional (paired)	0
Conditional (params)	1
Maximum edit distance	0
Fraction of missing alignments given 2% uniform base error rate	0.04
Maximum number of gap opens	1
Maximum number of gap extensions	-1
Disallow long deletion within [value] bp towards the 3'-end	16
Disallow insertion/deletion within [value] bp towards the end	0
Number of first subsequences to take as seed	-1
Maximum edit distance in the seed	2
Mismatch penalty	3
Gap open penalty	5
Gap extension penalty	4
Proceed with suboptimal alignments even if the top hit is a repeat	False
Disable iterative search	False
Maximum number of alignments to output in the XA tag for reads paired properly	3
Maximum number of alignments to output in the XA tag for disconcordant read pairs (excluding singletons)	10
Maximum insert size for a read pair to be considered as being mapped properly	500
Maximum occurrences of a read for pairing	100000
Quality threshold for read trimming down to 35bp	0

Table S4 Freebayes Parameters

Input Parameter	Value
Bam Alignment File	passaged
Additional Bam Alignment File	ancestor
Select Reference Genome	w303_draft
Freebayes Settings to Use	full
Theta	0.001
Ploidy	1
Pooled	False
Probability of variant threshold	0.0001
Show Reference Repeats	False
Ignore SNP alleles	False
Ignore insertion and deletion alleles	False
Ignore multi-nuceotide polymorphisms, MNPs	False
Ignore complex events (composites of other classes)	False
Use Best N Alleles	0
Left align indels	True
Base alignment quality (BAQ) adjustment	True
Use Reference Allele	False
Reference Ploidy	Haploid
Assign mapping quality of Q to the reference allele at each site	100
Reference Base Quality	60
Use duplicate reads	False
Minimum Mapping Quality	30
Minimum Base Quality	20
No Filters	True
Indel Exclusion Window	-1
Minimum Alternative Fraction	0.0
Minimum Alternative Count	1
Minimum Alternative Total	1
Minimum Coverage	0
Posterior Integration Limit N	1
Posterior Integration Limit M	3

Table S5 Unique mutations in the *msh2*Δ ancestor

Chr	Position	Original	Change	Gene	Consequence	Description of gene from SGD (Cherry *et al.* 1997)
chr03	196894	C	A	*PHO87*	missense D>Y	Low-affinity inorganic phosphate (Pi) transporter; involved in activation of PHO pathway; expression is independent of Pi concentration and Pho4p activity; contains 12 membrane-spanning segments; *PHO87* has a paralog, *PHO90*, that arose from the whole genome duplication
chr04	32896	C	G	*PHO13*	missense Q>E	Alkaline phosphatase specific for p-nitrophenyl phosphate; also has protein phosphatase activity
chr04	673259	T	TA	*TRS85*	frameshift	Subunit of TRAPPIII (transport protein particle), a multimeric guanine nucleotide-exchange factor for Ypt1p, required for membrane expansion during autophagy and the CVT pathway; directs Ypt1p to the PAS; late post-replication meiotic role
chr05	349345	AT	A	intergenic		
chr07	106429	G	T	*CHC1*	missense T>K	Clathrin heavy chain, subunit of the major coat protein involved in intracellular protein transport and endocytosis; two heavy chains form the clathrin triskelion structural component; the light chain (*CLC1*) is thought to regulate function
chr07	395930	TA	T	intergenic		
chr07	609723	GA	G	intergenic		
chr07	798380	AT	A	intergenic		
chr10	164291	AT	A	*AIM23*	frameshift, lose 4 amino acids	Mitochondrial translation initiation factor 3 (IF3, mIF3); evolutionarily conserved; binds to E. coli ribosomes in vitro; null mutant displays severe respiratory growth defect and elevated frequency of mitochondrial genome loss
chr12	340,658	C	T	*ICT1*	missense E>K	Lysophosphatidic acid acyltransferase; responsible for enhanced phospholipid synthesis during organic solvent stress; null displays increased sensitivity to Calcofluor white; highly expressed during organic solvent stress; *ICT1* has a paralog, *ECM18*, that arose from the whole genome duplication
chr15	92494	TA	T	*RPS19A*	frameshift within intron	Protein component of the small (40S) ribosomal subunit; required for assembly and maturation of pre-40 S particles; homologous to mammalian ribosomal protein S19, no bacterial homolog; mutations in human RPS19 are associated with Diamond Blackfan anemia; *RPS19A* has a paralog, *RPS19B*, that arose from the whole genome duplication
chr15	123982	CT	C	intergenic		
chr15	137881	TA	T	intergenic		
chr15	659986	G	T	*ALE1*	nonsense Y>stop	Broad-specificity lysophospholipid acyltransferase, part of MBOAT family of membrane-bound O-acyltransferases; key component of Lands cycle; may have role in fatty acid exchange at sn-2 position of mature glycerophospholipids
chr15	825192	G	A	intergenic		

Table S6 Mutation Spectra of Missense Variants

Functional Domain	Relevant Genotype	Single Base Pair Substitutions	Insertions or Deletions Di-or Tri Nucleotides	Insertions or Deletions Homopolymers	*p* value compared to null
Null	*msh2*Δ	7	6	134	0.47
Structural Integrity	*msh2-A618V*	8	11	98	0.02
	msh2-R657G	6	7	135	0.29
	msh2-L183P	7	12	131	0.03
	*msh2-C195Y**	15	7	151	0.81
	msh2-C345F	16	12	168	0.34
	*msh2-D621G**	12	5	139	0.83
	msh2-P640T	10	8	117	0.59
DNA binding	*msh2-R542L*	4	3	132	0.05
	msh2-D524Y	14	13	137	0.04
ATPase	*msh2-G688D*	15	12	127	0.04
	msh2-G693R	9	12	134	0.07
	*msh2-S695P**	14	9	150	0.73
	msh2-S742F	9	13	143	0.04
	msh2-T743K	5	9	137	0.10
	msh2-G770R	7	7	140	0.40

*plasmid rearrangement, data combined with *msh2*Δ data for the null control in Fisher Exact tests

References for Supplementary Material

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Cherry, J. M., C. Ball, S. Weng, G. Juvik, R. Schmidt *et al.*, 1997 Genetic and physical maps of *Saccharomyces cerevisiae*. Nature **387:** 67-73.

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